MRID No. 422981-02

DATA EVALUATION RECORD

1. **CHEMICAL:** Thiophanate-methyl
Shaughnessey No. 102001.

2. **TEST MATERIAL:** 1) Thiophanate-methyl (Topsin-M); Lot No. TIF-01016; 97.57% active ingredient; an off-white powder. 2) ^{14}C-Thiophanate-methyl; Lot No. Amersham, CFQ.4519GTW/C2/52; radiopurity of 95.3% and chemical purity of 90.1%.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants-Tier 2. Species Tested: *Anabaena flos-aquae*.

4. **CITATION:** Hoberg, J.R. 1991. (Thiophanate-methyl) - Toxicity to the Freshwater Blue-Green Alga *Anabaena flos-aquae*. SLI Report No. 91-10-3963. Conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by Atochem North America, Inc., Philadelphia, PA. EPA MRID No. 422981-02.

5. **REVIEWED BY:**

Heather Mansfield, Zoologist, Section 2
Ecological Effects Branch
Environmental Fate and Effects Division

Signature: Heather Mansfield
3/29/93

6. **APPROVED BY:**

Norman J. Cook, Head, Section 2
Ecological Effects Branch
Environmental Fate and Effects Division

Signature: Norman J. Cook
04-20-93

7. **CONCLUSIONS:**

This study is scientifically sound and meets the requirements for a Tier 2 aquatic plant growth and reproduction study. Both the EC_{50} and LOEC of *Anabaena flos-aquae* exposed to thiophanate-methyl were > 4.3 based on mean measured concentrations. The NOEC was 4.3 ppm. Although a precise EC_{50} was not found, the maximum dose tested is approximately 4x higher than the concentration expected from direct application to a 6" water column with the maximum application rate of 1.4 lbs a.i./acre.

8. **RECOMMENDATIONS:** N/A

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5. **REVIEWED BY:**

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Agronomist
KBN Engineering and
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6. **APPROVED BY:**

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Date: 7/16/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature:
Date:

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for a Tier 2 aquatic plant growth and reproduction study. The 5-day EC_{50} for *A. flos-aquae* exposed to thiophanate-methyl was >4.3 mg ai/l based on mean measured concentrations. The NOEC and LOEC were 2.2 and 4.3 mg ai/l, respectively.

8. **RECOMMENDATIONS:** N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Species: The alga used in the test, *Anabaena flos-aquae*, came from laboratory stock cultures originally obtained from Carolina Biological Supply Company, Burlington, NC. Stock cultures were maintained in Marine Biological Laboratory (MBL) medium (Table 1, attached) under test conditions. Transfers were made to fresh medium once weekly. The culture used as the inoculum for this test was transferred to fresh medium seven days before test initiation.

B. Test System: Test vessels were sterile 125-ml flasks fitted with stainless steel caps which permitted gas exchange. The vessels were conditioned by rinsing with appropriate test solutions and 50 ml of the test or control solution were placed into each flask. The test medium was the same as that used for culturing with a pH of 7.5. Test vessels were maintained on an orbital shaker (shaking rate of 100 rpm) under continuous cool-white illumination (approximately 130-300 footcandles at the surface of the media) in an environmental chamber. The temperature in the chamber was maintained at 24-25°C.

C. Dosage: Five-day growth and reproduction test. Based on the results of a preliminary test, six nominal concentrations of 0.16, 0.32, 0.64, 1.3, 2.5, and 5.0 mg active ingredient (ai)/l were selected for the definitive test.

A 62 µl aliquot (in acetone) of a 0.455 mg ai/ml ^{14}C-thiophanate-methyl solution was added to 500 ml of a 4.96 mg ai/l non-radiolabeled thiophanate-methyl (in MBL medium) solution. An additional 38 µl of acetone was added and this solution was diluted to 1 l with MBL medium to prepare the highest test solution (5.0 mg ai/l). Lower concentration test solutions were created by addition of appropriate volumes of the highest test solution to nutrient medium. All solutions contained 0.1 ml of acetone/l of nutrient solution. The test solutions were sonicated and stirred for 10 minutes each. A medium and solvent (0.1 ml acetone/l) control were also prepared.

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D. **Test Design:** The test consisted of 3 replicate flasks per treatment level and controls. An inoculum of *Anabaena flos-aquae* cells calculated to provide 3,000 cells/ml was aseptically introduced into each flask within one hour of solution addition. The inoculum volume was 510 µl per flask. At each 24-hour interval, cell counts were conducted on each replicate vessel using a hemacytometer and compound microscope. Terminal cell counts were conducted after sonicating the solutions for 60 seconds.

The conductivity and pH were measured at test initiation and termination. Temperature was recorded continuously with a minimum/maximum thermometer. The shaking rate of the orbit shaker was recorded daily. The light intensity was measured at the beginning of the test and every 24-hour interval of the exposure period.

At test initiation and termination, samples were removed from each test solution and the controls for analysis by liquid scintillation counting. Three quality control (QC) samples were prepared at test initiation and termination to judge the precision of the analyses.

E. **Statistics:** Since a clear dose-response was not observed after 120 hours of exposure, the EC_{50} was not determined.

The no-observed-effect concentration (NOEC) was determined using one-way analysis of variance (ANOVA) coupled with Bonferroni's test. The data were checked for normality using the Chi-square test and for homogeneity of variance using Hartley's test before conducting ANOVA.

12. **REPORTED RESULTS:** The mean measured concentrations of the test solutions were 0.14, 0.28, 0.55, 1.1, 2.2, and 4.3 mg ai/l which averaged 87% of nominal concentrations (Table 2, attached).

Cell densities determined at each observation time are presented in Table 3 (attached). Two replicates of the nutrient control were inadvertently not inoculated. The one remaining replicate was pooled with the three solvent control replicates for statistical analysis.

By test termination, mean cell densities in the treatments ranged from 56 to 161 x 10^4 cells per ml. Some cellular fragments were observed in the highest concentration solution (4.3 mg ai/l). Because no reduction in cell density was greater or equal to 50%, the EC_{50} was determined to be >4.3 mg ai/l. Consequently, the NOEC was 4.3 mg ai/l. Since this test was conducted above the maximum required exposure concentration (1.0 mg ai/l), no further testing to determine exposure-response was required.

Conductivity ranged from 220 to 230 µmhos/cm. The pH was between 7.1 and 7.6 in all test solutions and the controls at test initiation and between 7.7 and 8.2 at termination. The temperature ranged from 24 to 25°C during the study.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
No conclusions were made by the study author.

The study director confirmed that this study was conducted in compliance with EPA Good Laboratory Practice (GLP) regulations (40 CFR Part 160) with the exception that stability, characterization, and verification of test substance identity and maintenance of records on the test substance are the responsibility of the test sponsor. Additionally, routine water analyses were conducted by an independent laboratory that did not collect data in accordance with GLPs. The study was inspected by the Quality Assurance Unit of the testing laboratory on several occasions.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except that the light intensity during the test [1400-3240 lux (1 footcandle = 10.8 lux)] was occasionally higher or lower than recommended (2000 lux).

B. **Statistical Analysis:** Since none of the treatment concentrations inhibited the algal growth, the reviewer concurs with the author that the EC_{50} is above 4.3 mg ai/l. The reviewer used ANOVA coupled with Dunnett's test to determine the lowest-observed-effect concentration (LOEC) and NOEC in comparison to the solvent control data (see attached printout). The results from Dunnett's analysis indicated that the NOEC and LOEC were 4.3 and >4.3 mg ai/l, respectively.

Cellular fragmentation was observed in the 4.3 ppm solution, but this was not statistically significant.

C. **Discussion/Results:** This study is scientifically sound and meets the requirements for a Tier 2 non-target growth and reproduction test for aquatic plants. The 5 day NOEC, LOEC, and EC_{50} for *Anabaena flos-aquae* exposed to thiophanate-methyl were 4.3, > 4.3, and > 4.3 mg a.i./l, based on mean measured concentrations, respectively.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 3/29/93.

Table 1. **Composition of algal growth medium (MBL Medium) used in this study.**

Compound	Concentration (mg/L)
$CaCl_2 \cdot 2H_2O$	36.76
$MgSO_4 \cdot 7H_2O$	36.97
$NaHCO_3$	12.60
K_2HPO_4	8.71
$NaNO_3$	85.01
$FeCl_3 \cdot 6H_2O$	3.15
$CuSO_4 \cdot 5H_2O$	0.01
$CoCl_2 \cdot 6H_2O$	0.01
$ZnSO_4 \cdot 7H_2O$	0.022
$MnCl_2 \cdot 4H_2O$	0.18
$Na_2MoO_4 \cdot 2H_2O$	0.006
H_3BO_3	1.00
$Na_2EDTA \cdot 2H_2O$[a]	4.36

[a] The concentration of $Na_2EDTA \cdot 2H_2O$ in culture media was 4.36 mg/L. The concentration of EDTA included in media used in the toxicity test was 0.38 mg/L (0.384 mg $Na_2EDTA \cdot H_2O$/L).

Source: H.W. Nichols. 1973. Growth media - freshwater. pp.7-24; IN J. Stein, ed., *Handbook of Phycological Methods. Culture Methods and Growth Measurements.* Cambridge University Press.

Table 2. Concentrations of Thiophanate-methyl used in the 120-hour toxicity test with *Anabaena flos-aquae*.

Nominal Concentration (mg A.I./L)		Measured Concentration (mg A.I./L)[a]		
		0-Hour	120-Hour[b]	Mean
5.0	A	4.3	3.4	4.3(0.053)
	B	4.2	4.2	
	C	4.2	4.3	
2.5	A	2.1	2.1	2.2(0.047)
	B	2.2	2.0	
	C	2.1	2.1	
1.3	A	1.1	1.1	1.1(0.089)
	B	1.1	1.1	
	C	1.1	1.1	
0.64	A	0.54	0.52	0.55(0.024)
	B	0.54	0.59	
	C	0.53	0.54	
0.32	A	0.28	0.27	0.28(0.0066)
	B	0.28	0.29	
	C	0.28	0.28	
0.16	A	0.14	0.14	0.14(0.0023)
	B	0.13	0.14	
	C	0.14	0.14	
Control	A	<0.014	<0.014	
	B	<0.014	<0.014	
	C	<0.014	<0.014	
Solvent Control	A	<0.014	<0.014	
	B	<0.014	<0.014	
	C	<0.014	<0.014	
QC#1[c]		3.01(2.73)[d]	1.59(1.36)	
QC#2		3.98(3.64)	2.12(1.82)	
QC#3		4.97(4.55)	2.63(2.27)	

a Measured values are based on the unrounded analytical results and not on rounded numbers presented in this table.
b Test solutions sonicated for 60 seconds before analysis.
c QC = Quality Control sample
d Value in parentheses represents nominal fortified concentration.

Table 3. Cell density (x 10^4 cells/mL) of *Anabaena flos-aquae* after 24, 48, 72, 96 and 120 hours of exposure to Thiophanate-methyl.

Mean Measured Concentration (mg A.I./L)		OBSERVATION INTERVAL (HOURS) 24	48	72	96	120[a]	% Inhibition
4.3	A	0	0	0	109	33	
	B	0	0	13	212	152	
	C	0	0	0	23	112	
	Mean(SD)[b]	0(0)	0(0)	4(7)	115(95)	99(60)	—
2.2	A	0	0	48	13	51	
	B	0	33	0	19	65	
	C	0	11	64	513	151	
	Mean(SD)	0(0)	15(17)	37(33)	182(287)	89(54)	–
1.1	A	0	0	20	10	63	
	B	0	0	47	14	54	
	C	0	0	8	37	86	
	Mean(SD)	0(0)	0(0)	25(20)	20(15)	68(16)	–
0.55	A	0	0	0	11	144	
	B	0	0	11	52	21	
	C	0	0	19	16	83	
	Mean(SD)	0(0)	0(0)	10(10)	26(22)	82(61)	–
0.28	A	0	0	11	202	214	
	B	0	5	14	96	173	
	C	0	0	52	31	95	
	Mean(SD)	0(0)	2(3)	26(23)	109(86)	161(61)	—
0.14	A	0	0	41	17	54	
	B	0	0	7	18	58	
	C	0	3	39	79	55	
	Mean(SD)	0(0)	1(2)	29(19)	38(36)	56(2)	–
Control	A	NA[c]	NA	NA	NA	NA	
	B	NA	NA	NA	NA	NA	
	C	0	1	12	148	54	
	Mean(SD)[d]	-	-	-	-	-	
Solvent Control	A	0	0	18	18	60	
	B	0	0	32	140	56	
	C	0	1	8	7	46	
	Mean(SD)	0(0)	0.2(0.3)	19(12)	55(74)	54(7)	
Pooled Control[e]		0(0)	0.4(0.6)	18(11)	78(76)	54(6)	

a At test termination, 120 hours, all test solutions were sonicated for 60 seconds to disperse cell clumps and improve the accuracy of the cell counts. Solutions were not sonicated prior to test termination to prevent cell damage. Consequently, cell counts at 24-, 48-, 72- and 96-hours may not be an accurate representation of actual cell densities.
b Mean values are based on the unrounded data and not from the rounded results presented in this table.
c NA - Replicate vessels A and B were inadvertently not inoculated with algae.
d Mean could not be calculated from one replicate.
e Pooled control (replicate C) and solvent control (replicates A, B and C) data.

Anabaena cell density

Summary Statistics and ANOVA

Transformation = None

Group (Concentration mg ai/l*)	n	Mean	s.d.	cv%
1 = control	4	54.0000	5.8878	10.9
2 0.14	3	55.6667	2.0817	3.7
3 0.28	3	160.6667	60.4511	37.6
4 0.55	3	82.6667	61.5007	74.4
5 1.1	3	67.6667	16.5025	24.4
6 2.2	3	90.0000	53.1131	59.0
7 4.3	3	99.0000	60.5558	61.2

NOEC = 4.3 mg ai/l*
LOEC = >4.3 mg ai/l*
Numerous cellular fragments in highest test solutions indicate that the NOEC of 2.2 mg ai/l* LOEC = 4.3 mg ai/l* Not statistically significant

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by a t - test with Bonferroni adjustment of alpha level

* Based on mean measured concentrations

Minumum detectable difference for
t-tests with Bonferroni adjustment = -83.054241
This difference corresponds to -153.80 percent of control

```
************************************************
*                                              *
* Note - the above value for the minimum       *
* detectable difference is approximate as      *
* the sample sizes are not the same for all of *
* the groups.                                  *
*                                              *
************************************************
```

Between groups sum of squares = 25174.651515 with 6 degrees of freedom.

Error mean square = 1900.444444 with 15 degrees of freedom.

Bartlett's test p-value for equality of variances = .003

```
************************************************
*                                              *
* Warning - the test for equality of variances *
* is significant (p less than 0.01). The       *
* results of this analysis should be inter-    *
* preted with caution.                         *
*                                              *
************************************************
```